THE CHEMISTRY OF THE PHOSPHATE ESTERS OF INOSITOL

BY

MACK HAROLD McCORMICK

B.S., University of Kansas, 1948

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
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ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. H. E. Carter for his advice and friendly interest throughout this investigation.

The author also wishes to thank Mr. E. H. Burton for the X-ray work, and Miss Evelyn Petersen for the infrared spectra obtained in this work. He also wishes to thank the Upjohn Company for a generous grant in support of part of this work.

R. UNIV. BIBLIOTHEEK LEIDEN

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Introduction

Anderson first isolated crystalline inositol monophosphate from wheat bran in 1914 (1). Since then the ester has been reported as an enzymatic degradation product of sodium phytate (2), and in soybean phosphatide (3, 4). Recently, Iselin (5) has synthesized inositol-5-phosphate.

The probable presence of inositol monophosphate as a basic structure in inositol lipids of plant phosphatides (soybean, corn, linseed) stimulated our interest in obtaining a quantity of crystalline inositol monophosphate and in studying, in some detail, its physical, chemical and biological properties.

Discussion of Results

A reproducible method has been developed for the isolation of crystalline inositol monophosphate from a wheat bran hydrolysis of sodium phytate. The inositol monophosphate was separated from other phosphates by virtue of the solubility of its barium salt and the insolubility of its lead salt. The free acid was obtained by a cation exchange resin decomposition of the lead salt and crystallization of the inositol monophosphate from aqueous-alcohol. This phosphate has been characterized by the preparation of three crystalline derivatives (pentaacetate, brucine salt, dibenzylamine salt), its infrared spectrum and X-ray pattern. The $p\mathrm{K}'_{a}$ values of inositol monophosphate from phytate were determined by an electrometric titration of the free acid. The values ($p\mathrm{K}'_{a1} = 0.75$ and $p\mathrm{K}'_{a_2} = 5.68$) are very similar to those of glucose-3(or-4)-phosphate. *Acetobacter suboxydans* did not oxidize this inositol monophosphate.

A sample of inositol monophosphate isolated from soybean inositol lipid (6) has been crystallized by the same procedure used for the crystallization of inositol monophosphate from phytate.

It has been characterized by its infrared spectrum and X-ray pattern. *A. suboxydans* did not oxidize this phosphate.

A comparison of the infrared spectra and X-ray patterns of the inositol monophosphate from phytate and soybean inositol lipid shows the two compounds to be identical.

A comparison of the inositol-5-phosphate of Iselin (5) and inositol monophosphate from phytate (melting points of the free acids, pentaacetates of the acids, action of *A. suboxydans*) shows these two compounds to be identical. Therefore, the inositol-5-phosphate structure has been assigned to the inositol monophosphate from phytate and soybean inositol lipid.

The probable presence of inositol monophosphate has been demonstrated in an alkaline hydrolysis mixture of corn and linseed inositol lipids. This was accomplished with the use of paper chromatography of the mixture of phosphoric acids from the hydrolysis mixture.

The acetylation of epi-inosose reported by Posternak (7) to yield the pentaacetate of epi-inosose was found to yield as the initial crude material a hexaacetyl derivative (probably derived from the enol form of epi-inosose). This new product was isolated as a crystalline material (m.p. 138-140° C.). O-Acetyl values showed the presence of six O-Acetyl groups and the infrared spectrum failed to show the presence of a free keto group.

The pentaacetate of epi-inosose could be prepared by repeated recrystallization from absolute ethanol of the enol acetate. The infrared absorption spectrum indicated the presence of a free keto group.

Scyllo-*meso*-inosose, on acetylation, gives an acetyl derivative (m.p. 211°C.) which shows no keto group by infrared analysis. Treatment of this material with absolute ethanol gives a substance melting at 113-115°C. which seems to be identical with pentaacetyl-epi-inosose.

A mold *(Spicarea violacea)* has been found which will utilize inositol as the sole carbon source, completely replacing glucose. The enzyme poisons (iodoacetate, cyanide and floride) in the usual concentrations did not prevent growth of the mold. A three per

cent solution of sodium bisulfite completely inhibited the growth of the mold.

Summary

An improved method has been developed for the isolation of crystalline inositol monophosphate from sodium phytate. The product was characterized by the preparation of crystalline derivatives (pentaacetate, brucine salt, dibenzylamine salt), infrared spectrum, X-ray pattern and behavior toward *A. suboxydans.*

A crystalline sample of inositol monophosphate from soybean inositol lipid has been prepared. This inositol monophosphate and the one from phytate are identical (infrared spectrum, X-ray pattern, behavior toward *A. suboxydans*).

The inositol monophosphate from phytate and the inositol-5-phosphate of Iselin (5) have been compared. On the basis of the melting point of the free acids, the melting point of the pentaacetate and the action of *A. suboxydans* the two compounds appear to be identical.

The presence of inositol monophosphate in an alkaline hydrolysis mixture of the inositol lipid of corn and linseed lipids has been demonstrated.

The acetylation of epi-inosose has been shown to yield a hexaacetyl derivative probably derived from an enol acetate of epi-inosose.

The pentaacetate of scyllo-*meso*-inosose has been shown by infrared spectrum not to contain a free keto group.

A mold has been found *(S. violacea),* which will utilize inositol as its sole carbon source.

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VITA

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